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## ELEVATED PHENYLACETIC ACID LEVELS DO NOT CORRELATE WITH ADVERSE EVENTS IN PATIENTS WITH UREA CYCLE DISORDERS OR HEPATIC ENCEPHALOPATHY AND CAN BE PREDICTED BASED ON THE PLASMA PAA TO PAGN RATIO

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## Abstract

**Background**—Phenylacetic acid (PAA) is the active moiety in sodium phenylbutyrate (NaPBA) and glycerol phenylbutyrate (GPB, HPN-100), both are approved for treatment of urea cycle disorders (UCDs) - rare genetic disorders characterized by hyperammonemia. PAA is conjugated with glutamine in the liver to form phenylacetylgutamine (PAGN), which is excreted in urine. PAA plasma levels 500 µg/dL have been reported to be associated with reversible neurological adverse events (AEs) in cancer patients receiving PAA intravenously. Therefore, we have investigated the relationship between PAA levels and neurological AEs in patients treated with these PAA pro-drugs as well as approaches to identifying patients most likely to experience high PAA levels.

**Methods**—The relationship between nervous system AEs, PAA levels and the ratio of plasma PAA to PAGN were examined in 4683 blood samples taken serially from: [1] healthy adults [2], UCD patients 2 months of age, and [3] patients with cirrhosis and hepatic encephalopathy (HE). The plasma ratio of PAA to PAGN was analyzed with respect to its utility in identifying patients at risk of high PAA values.

**Results**—Only 0.2% (11) of 4683 samples exceeded 500 µg/ml. There was no relationship between neurological AEs and PAA levels in UCD or HE patients, but transient AEs including headache and nausea that correlated with PAA levels were observed in healthy adults. Irrespective of population, a curvilinear relationship was observed between PAA levels and the plasma PAA:PAGN ratio, and a ratio > 2.5 (both in µg/mL) in a random blood draw identified patients at risk for PAA levels > 500 µg/ml.

**Conclusions**—The presence of a relationship between PAA levels and reversible AEs in healthy adults but not in UCD or HE patients may reflect intrinsic differences among the populations and/or metabolic adaptation with continued dosing. The plasma PAA:PAGN ratio is a functional measure of the rate of PAA metabolism and represents a useful dosing biomarker.

### Keywords

BUPHENYL; glycerol phenylbutyrate; HPN-100; neurological adverse events; pharmacokinetics; RAVICTI; sodium phenylbutyrate

## INTRODUCTION

Glycerol phenylbutyrate, a sodium- and sugar-free phenylbutyrate derivative, and sodium phenylbutyrate are approved as ammonia lowering agents in patients with urea cycle disorders (UCDs). Both are pro-drugs of phenylacetic acid (PAA), which is formed by beta-oxidation from phenylbutyric acid (PBA) delivered either as glycerol phenylbutyrate following its intestinal hydrolysis by pancreatic lipases [1] or as sodium phenylbutyrate following dissociation in the stomach. PAA is conjugated with glutamine by glutamine-N-phenylacetyltransferase, largely in the liver and to a lesser extent in the kidney [2], to form phenylacetylglutamine (PAGN), which is excreted in urine, thereby providing an alternate pathway to urea for waste nitrogen excretion. In controlled studies population pharmacokinetic analyses of sodium phenylbutyrate and glycerol phenylbutyrate, it has been shown that the gastrointestinal absorption of PBA is approximately 75% slower when delivered as glycerol phenylbutyrate vs. sodium phenylbutyrate and that plasma PAA and PAGN levels show less variability during glycerol phenylbutyrate dosing. [3]-[7]. There are over 30 reports of the administration of sodium phenylacetate or sodium phenylbutyrate to healthy volunteers, patients with UCDs or other metabolic disorders and patients with cancer, many of which reported some adverse events (AEs) attributed to PAA (Supplemental Table 1) [8]-[36]. These reversible AEs in cancer patients were reported in studies involving continuous or intermittent intravenous administration designed to maintain high levels of PAA, suggesting that duration of exposure as well as peak PAA levels are important [35],[3].

The AEs reportedly associated with high levels of PAA have most commonly included nausea, headache, emesis, fatigue, weakness, lethargy, somnolence, dizziness, slurred speech, memory loss, confusion, and disorientation [35], [36]. Except for the symptoms of Kussmaul respiration, metabolic acidosis, cerebral edema, and coma associated with a fatal overdose of sodium phenylacetate/sodium benzoate (AMMONUL®)[13], the symptoms were rapidly reversible with reduced dosing or interruption of dosing.

Based on a detailed analysis of the timing of the AEs in relation to blood PAA concentrations, Simell calculated the safe upper PAA concentration limit to be 3.5 mmol/L, equivalent to 476 µg/mL [22], and Thibault reported that AEs were associated with PAA levels ranging from 499–1285 µg/mL [35], [36].

Sodium phenylbutyrate (BUPHENYL®) has been used for over three decades in the treatment of UCDs. Despite the fact that the AEs reportedly associated with elevated plasma PAA levels can mimic those associated with hyperammonemia, little is known regarding the relationship between PAA levels and AEs in UCD patients. The clinical trials of glycerol phenylbutyrate (RAVICTI®, HPN-100), which included over 100 UCD patients, 80 of whom underwent comparative study of sodium phenylbutyrate and glycerol phenylbutyrate [3] - [6] (the largest prospectively studied group of patients with this rare disorder), 193 patients with advanced cirrhosis complicated by hepatic encephalopathy (HE) [37], and more than 90 healthy adult subjects have afforded a unique dataset and opportunity to systematically examine the relationship between PAA levels and AEs and to explore biomarkers indicative of patients most likely to experience elevated PAA levels.

## METHODS

### Clinical Studies (Table 1)

Data from a thorough QTc study in healthy adults, five clinical studies in UCD patients and an open label safety and dose escalation study as well as a randomized, double-blinded controlled phase 2 study of patients with decompensated cirrhosis complicated by HE formed the basis for these analyses.

### UCD Patients

Eighty UCD patients completed 4 short-term (10 to 28 days) cross-over studies of sodium phenylbutyrate vs. glycerol phenylbutyrate (Table 1). The short-term UCD study population included 26 pediatric patients ages 2 mos through 17 years who received a mean (range) dose of 8 (1-19) g/day of glycerol phenylbutyrate or an equivalent dose of sodium phenylbutyrate and 54 adults patients ages 18 years or older who received a mean (range) dose of 13 (2-34) g/day of glycerol phenylbutyrate or an equivalent dose of sodium phenylbutyrate [3] [4] [5][6]. In addition, data from 100 UCD patients enrolled in 12-month glycerol phenylbutyrate treatment protocols including 49 children and 51 adults were analyzed in relation to PAA levels over time and the occurrence of the symptoms reported in cancer patients by Thibault [35][36][4] [5][6] during 12 months treatment.

### Patients with Cirrhosis and HE

Data from a 4-week safety and dose escalation study and a multicenter, randomized placebo-controlled study of 178 patients with cirrhosis and hepatic encephalopathy who received 13.2 g/day of glycerol phenylbutyrate (N=90) or placebo (N=88) for 16 weeks were analyzed [37], [38] (Table 1). Patients were monitored for safety and frequent PK samples were taken over the course of the study.

## Healthy Adults

A total of 98 healthy adults (mean age of 28; 53 male 45 female) participated in a blinded, randomized, cross over study to assess effects of glycerol phenylbutyrate and its metabolites on QTc and other ECG parameters (Table 1). In this protocol 12 subjects received 29.7 g/day, 4 subjects 39.6 g/day of glycerol phenylbutyrate and 68 subjects received placebo, moxifloxacin as the positive control and glycerol phenylbutyrate at doses of 13.2 g/day and 19.8 g/day administered three times daily for 3 days.

## Adverse Event Mapping

All treatment emergent adverse events (AEs) coded as to Body System as Nervous System Disorders using the Medical Dictionary for Regulatory Activities (MedDRA) in subjects enrolled in these studies were included in the analyses. For UCD patients, the specific toxicities reported by Thibault [35], [36], including nausea, headache, emesis, fatigue, weakness, lethargy, somnolence, dizziness, slurred speech, memory loss, confusion, and disorientation, exacerbation of neuropathy, pedal edema, hearing loss, abnormal taste, arrhythmia, rash, Kussmaul respiration, metabolic acidosis, increased anion gap, tachypnea, abdominal discomfort, cerebral edema, and obtundation or coma, were mapped to the MedDRA preferred terms in the clinical trial databases.

## Analysis of AEs in Relation to PAA Levels

Analyses were based on (a) 2126 samples from 98 healthy adults, (b) 1281 blood PAA and PAGN values derived from 80 UCD patients during the short term-switchover studies who received both sodium phenylbutyrate and glycerol phenylbutyrate, and (c) 428 samples from 90 patients with cirrhosis and HE who received glycerol phenylbutyrate. Because plasma PAA levels were not always available at the time the patient was experiencing an AE, the following rules were applied to associate an AE to a known PAA level. For healthy subjects, maximum PAA values recorded after the first dose but within 24 hours of the last dose and the incidence of neurological AEs (yes/no) were summarized by dosing period; for periods where subjects received placebo or moxifloxacin, the PAA levels were set to 0. For UCD patients, maximum PAA values (C<sub>max</sub>) recorded during each dosing period and the incidence of neurological AEs were summarized by treatment (glycerol phenylbutyrate or sodium phenylbutyrate). For HE patients, each AE was attributed to the PAA result that was closest in time to the AE.

The contribution of a 20 µg/mL increase in PAA levels to the probability of a neurological AE regardless of relationship to the study drug was examined using Generalized Estimating Equations [39]. For healthy subjects, data were summarized for each dose group. Since UCD patients received a range of doses, data were summarized for patients receiving a dose greater or less than the median dose (equivalent to 11.7 g/day). For HE patients, neurological AEs were examined both in relation to blinded treatment group assignment; i.e. glycerol phenylbutyrate or placebo, as well as in relation to PAA levels among patients treated with glycerol phenylbutyrate.

## Analysis of PAA in Relation to Plasma PAA:PAGN Ratio

GEE were used to model the predictive value of plasma PAA:PAGN ratio in identifying patients at risk of a high plasma PAA level as defined to have a PAA level equal or greater than 400 µg/mL or 500 µg/mL during 24 hours of dosing. Plasma PAA:PAGN ratios were grouped into binary categorical range of less than 2.5 or greater than 2.5. The repeated measures categorical outcome was modeled using GEE with a logit link function, ratio category as the independent variable, and the individual subject ID as the repeated measures factor. Confidence intervals for the predicted probabilities were computed by bootstrap estimation of 1000 re-samplings of the original data, as detailed in Davison and Hinkley [40].

## RESULTS

### UCD patients (Table 2, Figure 1)

Common AEs reported by at least 10% of patients during short-term treatment with either drug included diarrhea, flatulence, and headache. Neurological AEs reported by more than 1 UCD patient included headache, dizziness and dysgeusia. The mean (SD) PAA Cmax was similar in patients who reported at least one neurological AE, as compared with those who did not (50.8 (34.5) µg/mL vs 51.5 (49.23) µg/mL respectively). There was no statistically significant relationship in UCD patients between the presence or absence of neurological AEs and PAA levels during either glycerol phenylbutyrate or sodium phenylbutyrate treatment. The odds ratio of a neurological AE occurring for each 20 µg/mL increase in PAA levels for the two drugs combined, controlling for dose level, was 0.929, very close to 1 indicating that increasing levels of PAA were not associated with an increase in neurological AEs in these studies. There was no difference in the frequency of the PAA-associated AEs reported in cancer patients by Thibault [35], [36] in adult vs. pediatric UCD patients in the short-term controlled studies, despite the generally higher PAA levels in pediatric patients (Supplemental Table 2).

A total of 100 UCD patients enrolled in 12-month studies of glycerol phenylbutyrate received a mean (SD) total dose of 11.01 (5.970) g/day (range: 0.8–34.3 g). Overall common AEs reported in at least 10% of UCD patients during long-term treatment included vomiting, upper respiratory tract infection, nausea, nasopharyngitis, diarrhea, headache, hyperammonemia, decreased appetite, cough, fatigue, dizziness, and oropharyngeal pain. There was no increase either in plasma PAA levels (Supplemental Figure 1) or the rate of AEs over time. Just as in the short-term studies there was no difference between pediatric and adult patients in the frequency of the PAA-associated AEs reported in cancer patients by Thibault (Supplemental Table 2).

### Patients with cirrhosis and HE (Table 2, Figure 1)

Of 88 patients randomized to placebo, 48.9% reported a neurological AE as compared to 40.9% of 90 patients randomized to glycerol phenylbutyrate. Of the 428 PAA data points from patients randomized to glycerol phenylbutyrate, 46 were in patients who reported a neurological AE and 382 in patients who did not. The mean (SD) PAA value closest to occurrence of an AE was 61.4 (75.3) µg/mL while the mean PAA value not temporally



associated with an AE was 36.4 (55.6)  $\mu\text{g/mL}$  ( $p=0.77$ ) (Figure 2). Similar to UCD patients, there was no increase in the odds of experiencing a neurological AE with each 20  $\mu\text{g/mL}$  increment in PAA levels in cirrhosis patients (odds ratio 1.086;  $p=0.172$ ) indicating that at the dose of 13.2 g/day the odds of experiencing a neurological AE did not increase with an increase in PAA level.

### Healthy subjects

Common AEs in 10% of healthy volunteers included headache, nausea, and dizziness. Neurological AEs increased in frequency with increasing dose, ranging from 26.5% for 13.2 g/day to 91.7% for 29.7 g/day. Among those who reported a neurological AE, PAA values were higher for the 19.8 g/day, 29.7 g/day, and 39.6 g/day dosing periods than for the 13.2 g/day dosing period (Figure 2, Table 2). PAA levels increased as the dose of glycerol phenylbutyrate increased. In the case of the 13.2 g/day dose group, the difference was statistically significant (73.3 vs. 41.6,  $p < 0.001$ ) (Table 2). Logistic regression analysis indicated that each increment in PAA of 20  $\mu\text{g/mL}$  was associated with increasing odds of experiencing a neurological AE (odds ratio = 1.75;  $p = 0.006$ ). Individual AEs reported by healthy adults were generally transient and typically began within 36 hours of dosing and generally resolved with continued dosing, as depicted in Supplemental Figure 2.

### Plasma PAA:PAGN ratio as a Predictor of Elevated PAA Levels

PAA levels showed considerable variation over a 24-hr period in all patients regardless of the dose, drug and population (Figure 3). Unlike PAA, the ratio of PAA:PAGN was comparatively constant over 24 hours (data not shown). A curvilinear relationship was observed between PAA and PAA:PAGN in all populations, with a sharp upward inflexion beginning with PAA concentrations approaching 200  $\mu\text{g/mL}$  and a PAA:PAGN of approximately 2.5 or greater (Figure 4). Only 11 of a total of 4683 samples exceeded the 500  $\mu\text{g/mL}$  threshold level reported by Thibault to be associated with occurrence of neurological AEs in cancer patients. The estimated probabilities of correctly detecting a ratio 2.0 based on a single plasma sample taken at any time between the fasting morning sample (0 hr time point) and early evening (12 hr time point) remained relatively constant (77% to 84%), indicating that the timing of blood draw did not have an impact on the ratio of PAA:PAGN in plasma regardless of the PAA concentration. Patients with a ratio 2.5 had significantly higher PAA levels than those with a ratio 2.5 ( $p < 0.0001$ ) and PAA:PAGN ratios 2.5 had an approximately 20 times higher probability of being associated with PAA levels  $> 400 \mu\text{g/mL}$  (0.8% vs. 19.1%) or 500  $\mu\text{g/mL}$  (0.3% vs. 8.4%) (Table 3).

## DISCUSSION

No relationship was observed among UCD patients between PAA levels and either neurological AEs, or the specific AEs reported by Thibault, during treatment with either glycerol phenylbutyrate or sodium phenylbutyrate. This is supported by (a) the absence of a relationship during short term treatment in UCD patients, in which the odds ratio for the likelihood of a neurological AE for every 20  $\mu\text{g/mL}$  increase in PAA levels was 0.929, (b) the absence of a difference in the frequency of AEs similar to those reported in cancer patients by Thibault between pediatric and adult UCD patients during short or long-term

treatment, despite generally higher PAA levels in pediatric patients, and (c) the absence of any change in either PAA levels or the pattern of AEs during 12 months of dosing. Similarly, no statistical relationship was noted between PAA levels and neurological AEs among HE patients treated with 13.2g/day of glycerol phenylbutyrate for 16 weeks, as there was no difference in neurological AEs between the glycerol phenylbutyrate and placebo treatment arms, nor was there a relationship between PAA levels and the occurrence of neurological AEs.

Among the healthy adult volunteers, a relationship was observed between PAA levels and the occurrence of any neurological AE (e.g. headache, dizziness, vomiting and nausea). These AEs were generally mild, started early in the dosing period, and disappeared with continued dosing. The theoretical risk of PAA toxicity is expected to be similar for sodium phenylbutyrate or glycerol phenylbutyrate, as both drugs convert to PAA upon absorption. The AEs reported by healthy volunteers in these studies receiving glycerol phenylbutyrate are generally consistent with prior reports involving administration of sodium phenylbutyrate. The mechanism for these AEs is unknown, although interference with brain biochemical function has been suggested [41]. These differences between populations may be attributable either to metabolic differences between UCD and HE patients, who exhibit pathological nitrogen retention and high glutamine levels, as compared with healthy adults, and/or metabolic adaptation that may occur with continued exposure to PAA in chronically treated patients. Consistent with adaptation are the findings that AEs tended to disappear with continued dosing in healthy adults and that the UCD patients enrolled in these studies had been treated with sodium phenylbutyrate for an average of more than 9 years.

While most human tissues are capable of beta-oxidation and, hence, conversion of phenylbutyrate to PAA [42], enzymatic conversion of PAA to PAGN occurs primarily in the liver [2]. This may explain why conversion of PAA to PAGN appears to be a rate-limiting step in the metabolism of PAA prodrugs and why PAA metabolism may be compromised when liver function is poor, when availability of the precursor glutamine may be limited as in healthy subjects, and/or when the capacity of the enzymatic conversion may be limited as in very young children [7]. Regardless of the reason, decreases in the rate of PAGN formation are associated with an increased ratio of PAA to PAGN in plasma. It is interesting in this regard that the upward inflexion in PAA values assessed as a function of the PAA:PAGN ratio occurs at a concentration similar to the estimated  $K_m$  of this reaction based on population PK modeling, which is approximately 190  $\mu M$  as previously described by Monteleone et al [7].

In clinical practice, interpretation of an individual PAA value is compromised by the fact that concentrations vary considerably over the course of the day due to the relatively short half-lives of PBA and PAA. For example among the clinical trials comprising the present analyses, plasma PAA fluctuation index varied from 843% - 3931%; and fasting and maximal PAA levels in HE patients ranged from 0 - 1.3  $\mu g/mL$  and 248 - 532  $\mu g/mL$ , respectively. As compared with measurement of PAA alone, measurement of the PAA:PAGN ratio appears to be a useful proxy for the efficiency with which an individual patient converts PAA to PAGN, and a predictor of patients at risk of having an elevated PAA level. The PAA:PAGN ratio also has an important clinical advantage in that it remains



comparatively constant over the day and, therefore, is more readily interpretable in a random blood draw.

These analyses have several limitations. First, although pharmacokinetic and safety data were derived from controlled prospective studies, the analyses of the frequency of the specific AEs reported by Thibault et al. [35], [36] were done as post hoc analysis. Second, PAA levels were not always available at precisely the time of occurrence of neurological AEs, though a conservative approach was taken in these analyses by utilizing the highest recorded PAA for that dosing period. Finally, these conclusions pertaining to the absence of a statistical relationship between plasma PAA levels and neurological AEs apply at the population levels and may not apply to individual UCD or HE patients [38]. Since the symptoms reportedly associated with elevated PAA levels are non-specific and similar to those associated with elevated ammonia, it is possible that PAA may occasionally cause reversible AEs that go clinically unrecognized or are attributed to something else.

Collectively, the present findings indicate that the PAA:PAGN ratio is a useful dosing biomarker suitable for use with random blood draws and they suggest further that dose reduction may be warranted in patients receiving PAA prodrugs with an elevated plasma PAA:PAGN ratio who exhibit neurological adverse events not explained by elevated ammonia or intercurrent illness.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## List of Abbreviations

<b>GEE</b>	generalized estimating equations
<b>GPB</b>	glycerol phenylbutyrate (generic name for glyceryl tri (4-phenylbutyrate), also referred to as HPN-100 or RAVICTI®)
<b>HE</b>	hepatic encephalopathy
<b>NaPBA</b>	sodium phenylbutyrate (BUPHENYL®)
<b>PAA</b>	phenylacetic acid
<b>PAA:PAGN ratio</b>	ratio of the concentrations in µg/mL of PAA to PAGN in plasma
<b>PAGN</b>	phenylacetylglutamine
<b>PBA</b>	phenylbutyric acid
<b>SE</b>	safety extension
<b>SO</b>	switchover
<b>UCD</b>	urea cycle disorder

## REFERENCES

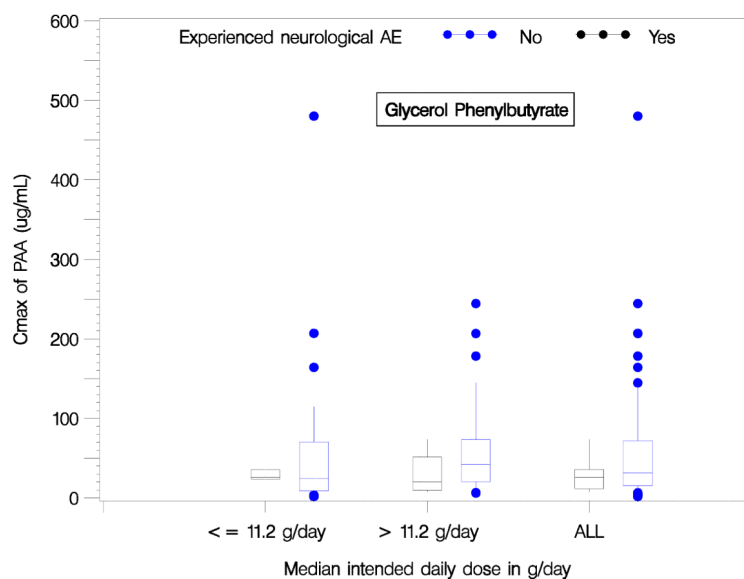
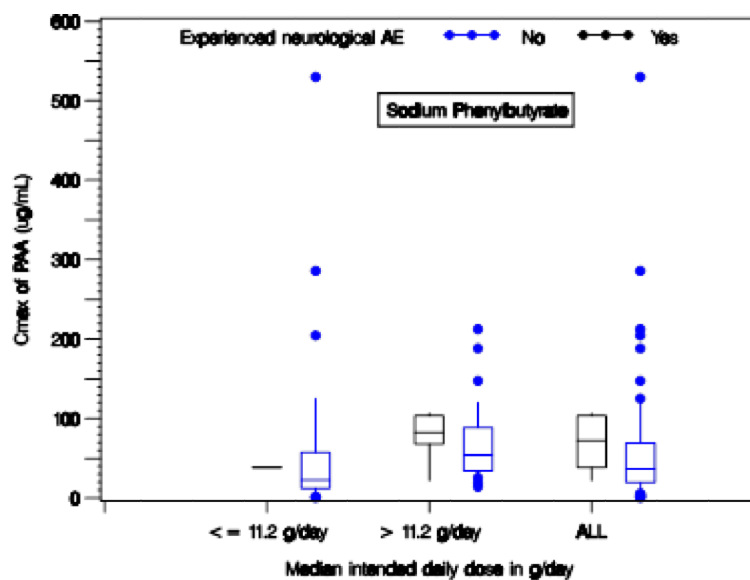
- McGuire BM, Zupanets I, Lowe ME, Sypliy V, Monteleone JP, Gargosky S, et al. Pharmacology and Safety of a Novel Ammonia Lowering Agents In Healthy Adults and Adults with Cirrhosis. *Hepatology*. 2010; 51:2077–85. [PubMed: 20512995]
- Moldave K. A Meister, Synthesis of phenylacetylglutamine by human tissue. *J. Biol. Chem.* 1957; 229:463–76. [PubMed: 13491597]
- Lee B, Rhead W, Diaz GA, Scharschmidt BF, Mian A, Shchelochkov O, et al. Phase 2 comparison of a novel ammonia scavenging agent with sodium phenylbutyrate in patients with urea cycle disorders: Safety, pharmacokinetics and ammonia control. *Mol. Genet. Metab.* 2010; 100:221–228. [PubMed: 20382058]
- Lichter-Konecki U, Diaz GA, Merritt II JL, Feigenbaum A, Jomphe C, Marier JF, et al. Ammonia control in children with urea cycle disorders (UCDs); Phase 2 comparison of sodium phenylbutyrate and glycerol phenylbutyrate. *Mol. Genet. Metab.* 2011; 103:323–329. [PubMed: 21612962]
- Diaz GA, Krivitzy L, Mokhtarani M, Rhead W, Bartley J, Feigenbaum A, et al. Ammonia control and neurocognitive outcome among urea cycle disorder patients treated with glycerol phenylbutyrate. *Hepatology*. 2013; 51:1271–1279.
- Smith W, Diaz GA, Lichter-Konecki U, Berry SA, Harding CO, McCandless SE, et al. Ammonia control in children ages 2 months through 5 years with urea cycle disorders (UCDs); Comparison of sodium phenylbutyrate and glycerol phenylbutyrate. *J. Pediatr.* 2013; 162:1228–1234. [PubMed: 23324524]
- Monteleone JPR, Dykstra KH, Lee B, McGuire BM, Berry S, Diaz G, et al. Glycerol phenylbutyrate, a novel investigational ammonia scavenging agent, demonstrated unique behavior based on pharmacokinetic / pharmacodynamic (PKPD) modeling. *J. Clin. Pharmacol.* 2013; 53:699–710. [PubMed: 23775211]
- MacArthur R, Altincatal A, Tuchman M. Pharmacokinetics of sodium phenylacetate and sodium benzoate following intravenous administration as both a bolus and continuous infusion to healthy adult volunteers. *Mol. Genet. Metab.* 2004; 81:S67–S73. [PubMed: 15050977]
- Ambrose AM, Power FW, Sherwin CP. Further studies on the detoxication of phenylacetic acid. *J. Biol. Chem.* 1933; 101:669–675.
- Sherwin C, Wolf M, Wolf W. The maximum production of glutamine by the human body as measured by the output of phenylacetylglutamine. *J. Biol. Chem.* 1919; 37:113–119.

11. Sherwin C, Kennard S. Toxicity of phenylacetyl acid. 1919; 40:259–264.
12. Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival after treatment with phenylacetate and benzoate for urea-cycle disorders. *New Engl. J. Med.* 2007; 356:2282–2292. [PubMed: 17538087]
13. Praphanphoj V, Voyadjiev SA, Waber LJ, Brusilow SW, Geraghty MT. Three cases of intravenous sodium benzoate and sodium phenylacetate toxicity occurring in the treatment of acute hyperammonaemia. *J. Inherit. Metab.* 2000; 23:129–136.
14. Brusilow SW. Arginine, an indispensable amino acid for patients with inborn errors of urea synthesis. *J. Clin. Invest.* 1984; 72:2144–2148. [PubMed: 6511918]
15. Batshaw ML, Thomas GH, Brusilow SW. New approaches to the diagnosis and treatment of inborn errors of urea synthesis. *Pediatrics.* 1981; 68:2, 290–297.
16. Brahe C, Vitali T, Tiziano FD, Angelozzi C, Pinto AM, Borgo F, et al. Phenylbutyrate increases SMN gene expression in spinal muscular atrophy patients. *Eur. J. Human Genet.* 2005; 13:256–259. [PubMed: 15523494]
17. Mercuri E, Bertini E, Messina E, Pelliccioni M, D'Amico A, Colitto F, et al. Pilot trial of phenylbutyrate in spinal muscular atrophy. *Neuromusc. Disord.* 2004; 14:130–135. [PubMed: 14733959]
18. Zeitlin PL, Diener-West M, Rubenstein RC, Boyle MP, Lee CKK, Brass-Ernst L. Evidence of CFTR function in cystic fibrosis after systemic administration of 4-phenylbutyrate. *Molecular Therapy.* 2002; 6:119–126. [PubMed: 12095312]
19. Rubenstein RC, Zeitlin PL. A pilot clinical trial of oral sodium 4-phenylbutyrate (Buphenyl) in F508-homozygous cystic fibrosis patients: partial restoration of nasal epithelial CFTR function. *Am. J. Respir. Crit. Care Med.* 1998; 157:484–490. [PubMed: 9476862]
20. Collins AF, Pearson HA, Giardina P, McDonagh KT, Brusilow SW, Dover GJ. Oral sodium phenylbutyrate therapy in homozygous thalassemia: A clinical trial. *Blood.* 1995; 85:43–49. [PubMed: 7528572]
21. Dover GJ, Brusilow S, Charache S. Induction of fetal hemoglobin production in subjects with sickle cell anemia by oral sodium phenylbutyrate. *Blood.* 1994; 84:339–343. [PubMed: 7517215]
22. Simell O, Siplia I, Rajante J, Valle D, Brusilow SW. Waste nitrogen excretion via amino acid acylation: Benzoate and phenylacetate lysinuric acid protein intolerance. *Pediatr. Res.* 1986; 20:1117–1121. [PubMed: 3099249]
23. Lin J, Gilbert J, Rudek MA, Zwiebel JA, Gore S, Jiemjit A, et al. A phase I dose-finding study of 5-azacytidine in combination with sodium phenylbutyrate in patients with refractory solid tumors. *Clinical Cancer Therapy.* 2009; 15:6241–6249.
24. Camacho LH, Olson J, Tong WP, Young CW, Spriggs DR, Malkin MG. Phase I dose escalation clinical trial of phenylbutyrate sodium administered twice daily to patients with advanced solid tumors. *Invest. New Drugs.* 2007; 25:131–138. [PubMed: 17053987]
25. Sung MW, Waxman S. Combination of cytotoxic-differentiation therapy with 5-fluorouracil and phenylbutyrate in patients with advanced colorectal cancer. *Anticancer Research.* 2007; 27:995–1002. [PubMed: 17465233]
26. Maslak P, Chaneil S, Camacho L, Soignet S, Pandolfi PP, Guernah I, et al. Pilot study of combination transcriptional modulation therapy with sodium phenylbutyrate and 5-azacytidine in patients with acute myeloid leukemia or myelodysplastic syndrome. *Leukemia.* 2006; 20:212–217. [PubMed: 16357841]
27. Thompson P, Balis F, Serabe BM, Berg S, Adamson P, Klenke R, et al. Pharmacokinetics of phenylacetate administered as a 30-min infusion in children with refractory cancer. *Cancer Chemother. Pharmacol.* 2003; 52:417–423. [PubMed: 12879279]
28. Gore SD, Weng LJ, Figg WD, Zhai S, Donehower RC, Dover G, et al. Impact of prolonged infusions of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. *Clin. Cancer Res.* 2002; 8:963–970. [PubMed: 11948101]
29. Burstein AH, Reed E, Tompkins AC, Venzon D, Figg WD. Phenylacetate pharmacokinetics based on iterative two-stage population analysis. *Pharmacotherapy.* 2001; 21:281–286. [PubMed: 11253852]

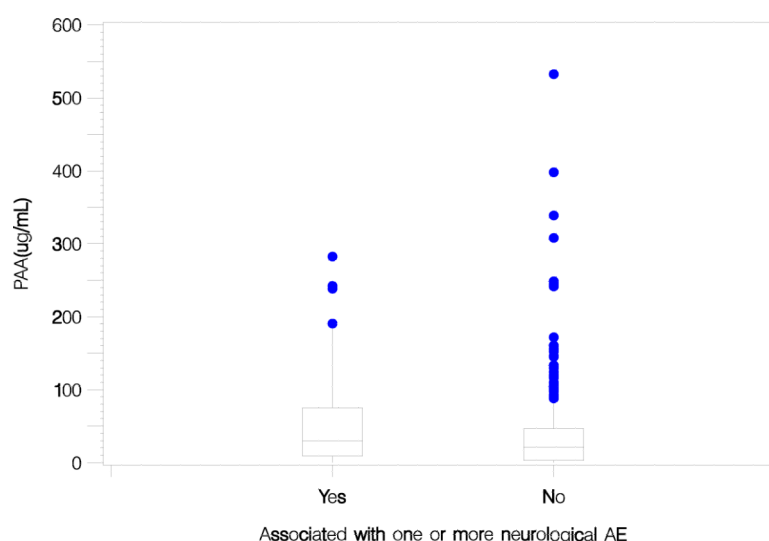
30. Carducci MA, Gilbert J, Bowling MK, Noe D, Eisenberger MA, Sinibaldi V, et al. A Phase I clinical and pharmacological evaluation of sodium phenylbutyrate on an 120-h infusion schedule. *Clin. Cancer Res.* 2001; 7:3047–3055. [PubMed: 11595694]
31. Gore SD, Weng LJ, Zhai S, Figgs WD, Donehower RC, Dover GJ, et al. Impact of the putative differentiating agent sodium phenylbutyrate on myelodysplastic syndromes and acute myeloid leukemia. *Clin. Cancer Res.* 2001; 7:2330–2339. [PubMed: 11489809]
32. Gilbert J, Baker SS, Bowling MK, Grochow L, Figg WD, Zabelina Y, et al. A phase I dose escalation and bioavailability study of oral sodium phenylbutyrate in patients with refractory solid tumor malignancies. *Clin. Cancer Res.* 2001; 7:2292–2300. [PubMed: 11489804]
33. Chang SM, Kuhn JG, Robins HI, Schold SC, Spence AM, Berger MS, et al. Phase II study of phenylacetate in patients with recurrent malignant glioma: A North American brain tumor consortium report. *J. Clin. Oncol.* 1999; 17:984–990. [PubMed: 10071293]
34. Piscitelli S, Thibault A, Figg W, Tompkins A, Headlee D, Lieberman R, et al. Disposition of phenylbutyrate and its metabolites, phenylacetate and phenylacetylglutamine. *J. Clin. Pharmacol.* 1995; 35:368–373. [PubMed: 7650225]
35. Thibault A, Samid D, Cooper MR, Figg WD, Tompkins AC, Patronas N, et al. Phase I study of phenylacetate administered twice daily to patients with cancer. *Cancer Research.* 1995; 75:2932–2938.
36. Thibault A, Cooper M, Figg W, Venzon D, Sartor A, Tompkins A, et al. Phase I and Pharmacokinetic Study of Intravenous Phenylacetate in Patients with Cancer. *Cancer Research.* 1994; 54:1690–1694. [PubMed: 8137283]
37. C Rockey D, Vierling JM, Mantry P, Ghabril M, Brown RS Jr, Alexeeva O, et al. Randomized, controlled, double blind study of glycerol phenylbutyrate in patients with cirrhosis and episodic hepatic encephalopathy. *Hepatology.* 2013 in press.
38. Ghabril M, Zupanets IA, Vierling J, Mantry P, Rockey D, Wolf D, et al. Glycerol phenylbutyrate in patients with cirrhosis and hepatic encephalopathy: A pilot study of safety and effect on ammonia. *Clin. Pharmacol. in Drug Development* (published online March, 2013) H.B. Mann, D.R. Whitney, On a test of whether one of two random variables is stochastically larger than the other. *Ann. of Math. Stat.* 1947; 18:50–60.
39. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika.* 1986; 73:13–22.
40. Davison AD, Hinkley CV. Bootstrap methods and their application. *Cambridge Series in Statistical and Probabilistic Mathematics.* 1997:358–362.
41. Potempska A, Loo YH, Wisniewski HM. On the possible mechanism of phenylacetate neurotoxicity: inhibition of choline acetyltransferase by phenylacetyl-CoA. *J. Neurochem.* 1985; 42:1499–1501. [PubMed: 6142928]
42. Kormanik K, Kang H, Cuebas D, Vockley J, Mohsen AW. Evidence for the involvement of medium chain acyl dehydrogenase in the metabolism of phenylbutyrate. *Mol. Genet. Metab.* 2012; 107:684–689. [PubMed: 23141465]

**Highlights**

- Plasma PAA levels > 500 ug/dl associated with neurological AE in cancer patients
- Investigated trend of PAA and neurological AE in patients treated with PAA pro-drug
- Neurological AEs are transient in patients treated with PAA pro-drug
- High plasma PAA/PAGN identified patients at risk for high PAA levels
- No correlation was found in patients between PAA levels and neurological AEs

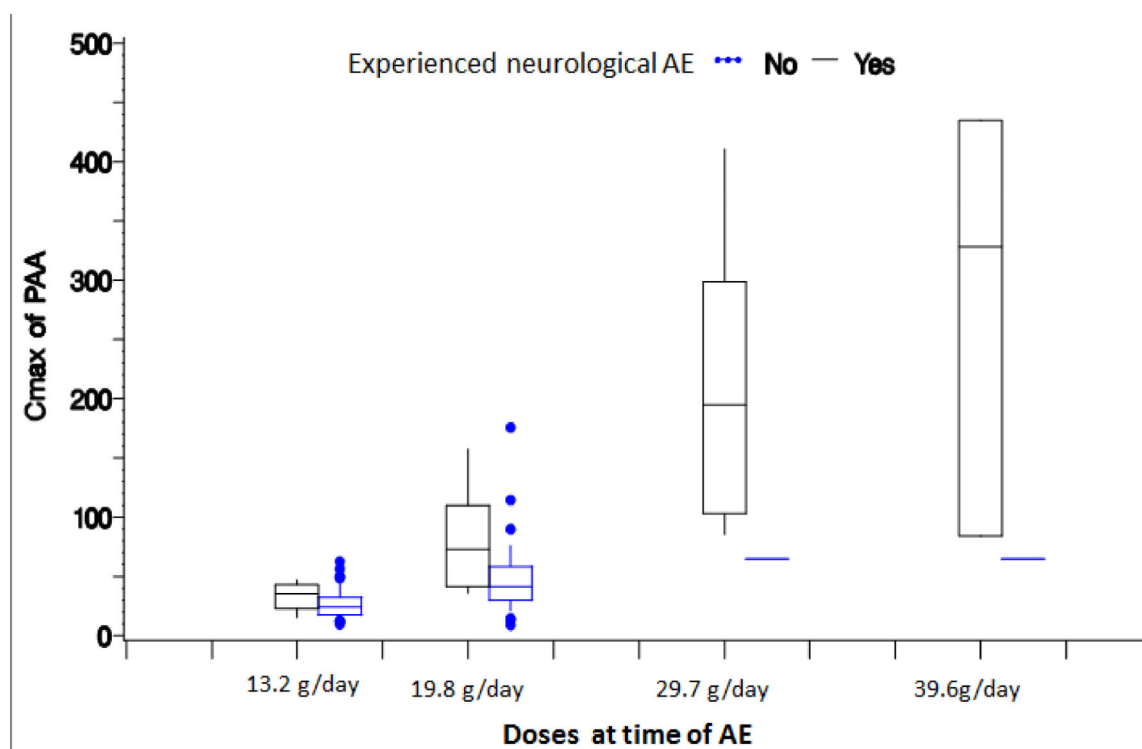






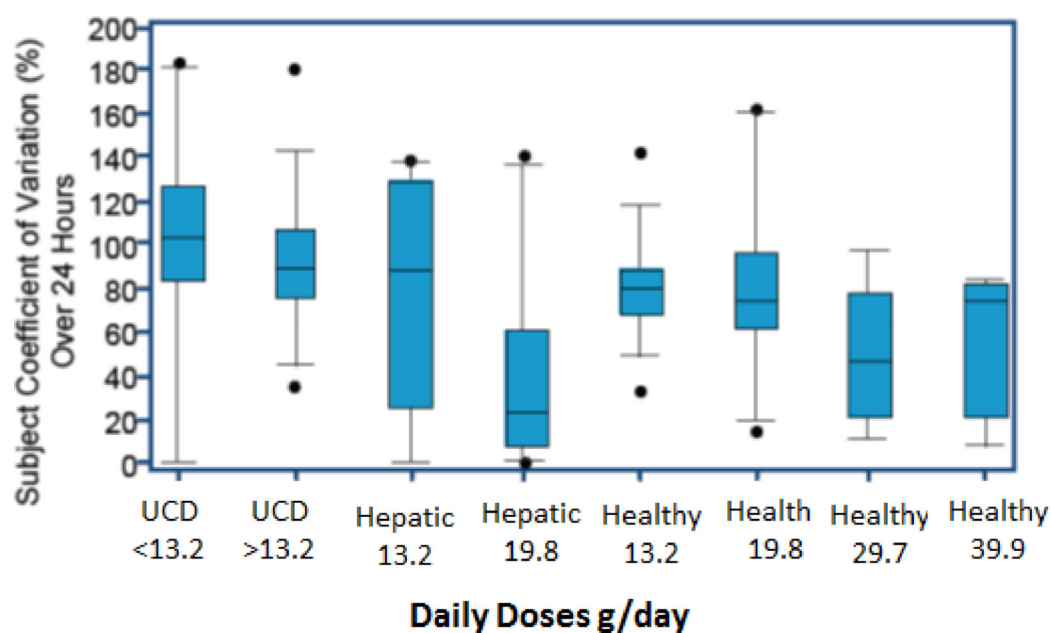
**Figure 1. Lack of Relationship Between PAA Levels and Neurological AEs in UCD and HE Patients**

The top and middle panels depict box-and-whisker plots for the mean maximal (C<sub>max</sub>) concentration of PAA during dosing of UCD patients with sodium phenylbutyrate and glycerol phenylbutyrate, respectively. There was no statistical difference in maximal PAA levels between UCD patients who did or did not report neurological AEs. The bottom panel depicts mean PAA concentrations (mean [SD] = 61.4 [75.3] vs. 36.4 [55.6];  $p = 0.77$ ) among patients with cirrhosis and hepatic encephalopathy randomized to treatment with glycerol phenylbutyrate who reported neurological adverse events. The range of PAA concentrations as reflected by the box (25<sup>th</sup> to 75<sup>th</sup> percentile) are similar for patients who did or did not report an AE. The dots depict individual values. (See Table 2 for statistical summary)



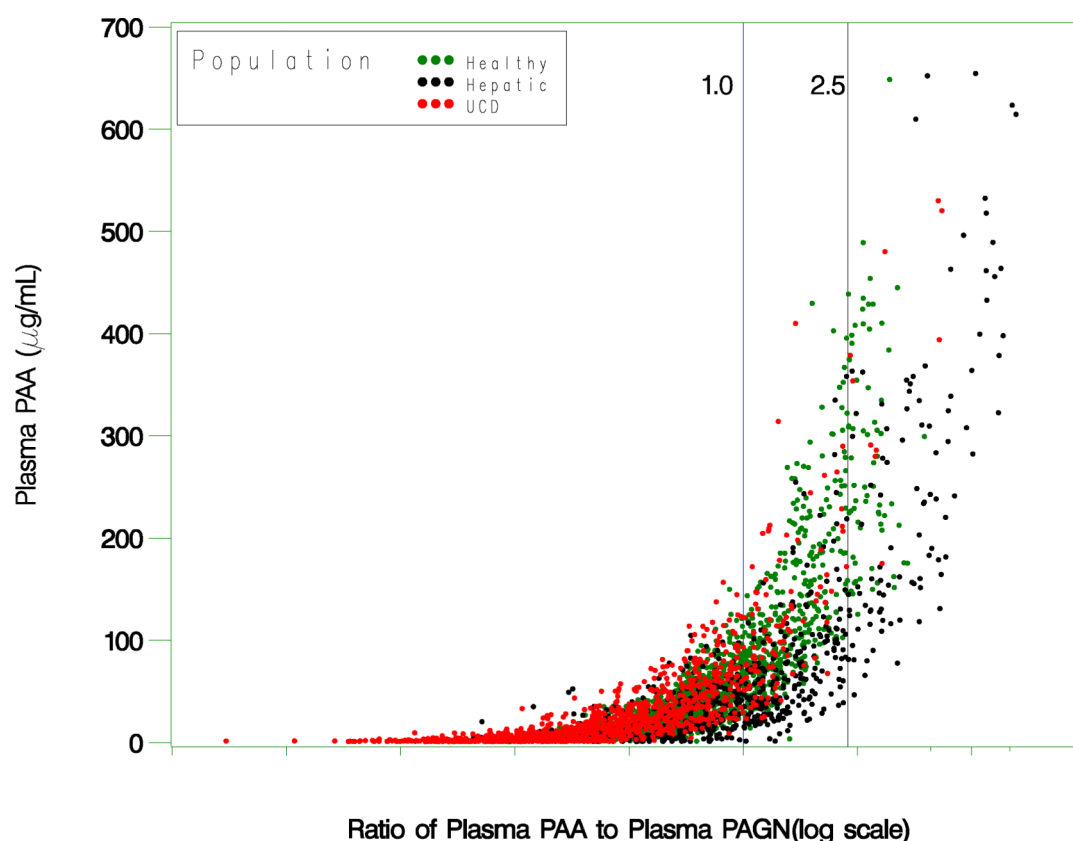
**Figure 2. PAA Levels in Healthy Adults Reporting a Nervous System Adverse Event (AE) Grouped by Dose**

The maximum PAA value (C<sub>max</sub>) is displayed in relation to dose of glycerol phenylbutyrate for patients who did or did not report a neurological adverse event (AE), regardless of relationship to study drug or timing relative to blood draw for PAA. The box and whisker plots depict mean (horizontal line), 25-75 percentiles (box) and 10 and 90% confidence intervals. Note that a wide range of PAA levels was observed at each dose and among patients with or without AEs. PAA levels were significantly higher among patients with AEs as compared to those without at the 6mL TID dose, but not at the 4 mL TID dose. All but 1 subject in the 9 and 12 mL dose groups reported a neurological AE. (See Table 2 for statistical summary)



**Figure 3. Plasma PAA Intra-subject Variability**

Healthy subjects and patients with UCD or HE underwent serial blood sampling over 12 to 24 hours. The figure depicts the coefficient of variation (CV%) as an indicator of intra-subject variability. Regardless of the dose or population, there is high degree of variability among all subjects.



**Figure 4. Plasma PAA vs. Plasma PAA:PAGN Ratio**

PAA levels in µg/mL (Y axis) are plotted in relation to the ratio of PAA to PAGN concentration (both expressed as µgµg/mL) in plasma (X axis) in that same sample. This plot includes >3500 samples from all populations, including healthy adults (Healthy), patients with cirrhosis and hepatic encephalopathy (Hepatic), and patients with urea cycle disorders (UCD). All populations exhibit a similar relationship, with the upward inflection point occurring at ratios exceeding approximately 2.5 and PAA concentrations in the range of 100-200 µg/ml.

**Table 1**

## Clinical Studies and Subject Disposition

Populations	Study ID, Design and Objectives	Ages / No. Treated	Study Drug / Duration
<b>Healthy Adult Volunteers</b>	<b>HPN-100-010</b> Thorough QT/QTc study Arm 1: safety run-in Arm 2: double-blind, randomized, crossover	Adults 18 N:98 Arm 1: 9 mL TID: 12 12 mL TID: 4 Arm 2: 4 mL TID: 68 6 mL TID: 68	GPB 3 days
<b>UCD Patients</b>	<b>UP 1204-003</b> Phase 2, open-label, fixed-sequence, switch-over study	Adults 18 N:10	Open label, fixed sequence, NaPBA to GPB switchover 7 days on each drug
	<b>HPN-100-006</b> Phase 3, randomized, double-blind, crossover, active-controlled, multiple-dose study	Adults 18 N: 44	GPB and NaPBA 14 days randomized, double blind, double dummy cross over
	<b>HPN-100-005SO</b> Phase 2, open-label, fixed-sequence, switch-over, multiple-dose study with 12-month safety extension	Pediatric, patients ages 6-17 N:11	Open label, fixed sequence, NaPBA to GPB switchover 7 days on each drug
	<b>HPN-100-012SO</b> Phase 3b, open-label, fixed-sequence, switch-over study	Pediatric, patients ages 2 months to < 6 yrs N:15	Open label, fixed sequence, NaPBA to GPB switchover 7 days on each drug
	<b>HPN-100-005SE</b> Phase 2, open-label 12-month safety-extension study	Pediatric ages 6 – 17 years N: 17	GPB 12 months
	<b>HPN-100-012SE</b> Phase 2, open-label 12-month safety-extension study	Pediatric ages 29 days to <6 years N: 23	GPB 12 months
	<b>HPN-100-007</b> Phase 3, open-label, , 12-month safety-extension study	Adult and pediatric ages 6 N: 60 (51 adults, 9 pediatric patients)	GPB 12 months
<b>HE Patients</b>	<b>HPN-100-008 (Part A) Open label, safety and dose-escalation study</b>	Adults 18 N: 15	GPB 4 weeks
	<b>HPN-100-008*</b> Randomized, double-blind, placebo-controlled phase 2 study	Adults 18 N: 178	GPB 16 weeks

GPB – glycerol phenylbutyrate; HE – hepatic encephalopathy; NaPBA – sodium phenylbutyrate; SE – safety extension; SO – switchover; UCD – urea cycle disorders.

\* Used for analysis of PAA levels in relation to AEs only.

Table 2

Analysis of the Relationship Between PAA and Neurological Adverse Events

Healthy Subjects			UCD Patients		HE Patients		
Dose	13.2 g/d	19.8 g/d	29.7 g/d	39.6 g/d	< = 11.7 g/d	> 11.7 g/d	13.2g/day
AE Reported							
	Yes	No	Yes	No	Yes	No	Yes
n	15	51	31	38	8	1	3
Plasma PAA (µg/mL)							
Mean	32.9	26.9	86.3	48.1	211.1	65.0	282.4
SD	13.1	12.2	53.9	30.2	118.4	--	179.5
Median	35.5	24.6	73.3	41.6	194.8	65.0	328.0
p-value <sup>a</sup>	0.076		<0.001		0.222		0.500
Odds ratio	1.75 <sup>b</sup>						
p-value <sup>d</sup>	0.006						
	0.929 <sup>c</sup>						
	0.529						
	0.935						
	0.77						
	1.086 <sup>c</sup>						
	0.172						

AE – adverse event; HE – hepatic encephalopathy; PAA – phenylacetic acid; UCD v urea cycle disorders

<sup>a</sup> p-value comparing subjects reporting a neurological AE within each dose group using an exact non- parametric Mann-Whitney test.

<sup>b</sup> Odds ratio of experiencing a neurological AE associated with a 20 µg/mL increase in PAA, C<sub>max</sub> controlling for dose group, including placebo and moxifloxacin, for which PAA was assumed to be zero.

<sup>c</sup> Odds ratio (p-value) of incidence of neurological AE associated with a 20-µg/mL increase in PAA C<sub>max</sub>.

<sup>d</sup> p-value for the odds ratio. UCD patient data are derived from studies UP-1204-003, HPN-100-005SO, HPN-100-006, and HPN-100-012SO. HE patient data are derived from study HPN-100-012 Part B only.



**Table 3**

Predictive Value of Plasma PAA:PAGN Ratio

Plasma PAA	Plasma PAA:PAGN Ratio	Probability of a Plasma PAA Value > 400 or 500 µg/mL
400 µg/mL	2.5	0.8%
	> 2.5	19.1%
>500 µg/mL	25	0.3%
	>2.5	8.4%

PAA – phenylacetic acid; PAGN – phenylacetylglutamine; PAA:PAGN ratio – ratio of the concentrations of PAA to PAGN in plasma, both in (µg/mL)